Title: Natural selection acts on Atlantic salmon major histocompatibility (MH) variability in the wild

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Summary

Pathogen driven balancing selection is thought to maintain polymorphism in Major Histocompatibility (MH) genes. However, there have been few empirical demonstrations of selection acting on MH loci in natural populations. To determine whether natural selection on MH genes has fitness consequences for wild Atlantic salmon in natural conditions, we compared observed genotype frequencies of Atlantic salmon (*Salmo salar*) surviving in a river six months after their introduction as eggs with frequencies expected from parental crosses. We found significant differences between expected and observed genotype frequencies at the MH class II alpha locus but not at a MH class I-linked microsatellite or at seven non-MH-linked microsatellite loci. We therefore conclude that selection at the MH class II alpha locus was a result of disease-mediated natural selection, rather than any demographic event. We also show that survival was associated with additive allelic effects at the MH class II alpha locus. Our results have implications for both the conservation of wild salmon stocks, and the management of disease in hatchery fish. We conclude that natural or hatchery populations have the best chance of dealing with episodic and variable disease challenges if MH genetic variation is preserved both within and among populations.

Key Words: MH (Major Histocompatibility), Natural Selection, Atlantic salmon

1. Introduction

The genes of the major histocompatibility complex (MHC) encode proteins that play a crucial role in the vertebrate immune response (Klein 1986) and several lines of evidence suggest that MHC polymorphism is maintained by pathogen-driven balancing selection (Hughes & Nei 1989; Hedrick et al. 2002). Possible mechanisms for this balancing selection include heterozygote advantage, negative frequency-dependent selection or varying pathogen resistance over space and time (Hedrick et al. 1999; Hedrick et al. 2002), and there is still much debate about which of these mechanisms is most relevant to MHC. MHC class I and class II alleles have been implicated in conferring disease resistance or susceptibility, but little empirical data is available (Sommer 2005). Associations between the variability at the human MHC (HLA) and disease susceptibility have been studied in great detail and have been shown for both autoimmune and infectious diseases (Hughes *et al.* 1996; Marsh *et al.* 2000). Studies in experimental models such as mice have demonstrated clear effects of MHC genotypes with regard to susceptibility to infections (McClelland et al. 2003; 2004). Investigations in natural vertebrate populations have focussed mainly on parasite-driven selection on MHC and in general, support the view that frequency-dependent selection in the form of rare allele advantage is the most important mechanism for maintaining MHC genetic diversity (Sommer 2005). Links between MH genes and resistance or susceptibility to several major salmonid diseases, such as furunculosis and infectious salmon anaemia, have recently been found in farmed Atlantic salmon (Langefors et al. 2001a; Lohm et al. 2002; Grimholt et al. 2003), but as yet, disease-mediated selection on natural populations of this species has not been demonstrated (Bernatchez & Landry 2002). The aim of this study, therefore, was to

empirically determine whether natural selection on MH genes has fitness consequences for wild Atlantic salmon in natural conditions and hence to assess whether disease-mediated selection could potentially impact on the genetic variability of wild populations.

The Atlantic salmon (*Salmo salar* L.) is an anadromous fish that spends the first part of its life in freshwater (typically one to four years) and a further one to three years feeding in the ocean, before returning to natal rivers to spawn. Atlantic salmon are well suited to studies of natural selection on MH loci because they dominantly express single MH class I, class II alpha and class II beta loci (Grimholt *et al.* 2002; Stet *et al.* 2002). Any natural selection is therefore targeted on fewer loci and genetic analysis is relatively simple compared to species with multiple loci of each MHC class. MH class I and II loci are not linked in this species (Grimholt *et al.* 2002), hence, selective pressures on both sets of proteins can be assessed independently. (As MHC genes in teleosts do not form a single complex they are therefore known simply as MH genes in this taxon (Stet *et al.* 2002)). Atlantic salmon are a useful experimental model species because they produce large numbers of progeny, enabling large sample sizes to be acquired subsequent to periods of high mortality, such as the period following emergence from nests (Einum & Fleming 2000). The understanding of the basis of disease and immune response in Atlantic salmon is critical for the conservation of wild stocks. It also has implications for the sustainable management of salmon aquaculture, and for potential interactions between wild and cultured fish (Naylor *et al.* 2005; Roberge *et al.* 2006). While direct genetic effects of introgression between wild and hatchery-reared salmon have been demonstrated (McGinnity *et al.* 2003), the impact of diseases originating from aquaculture (Håstein & Lindstad 1991;

Johnsen & Jensen 1994; McVicar 1997) on the genetic integrity of wild fish has not been addressed.

Natural selection resulting from disease should only be detectable at immunogenetic loci, such as MH, while other forces such as genetic drift, migration and mutation should be detectable at both control and immunogenetic loci (Garrigan & Hedrick 2003). The immunogenetic loci included in this study were: (a) *Sasa-UBA-3UTR*, a microsatellite marker embedded in the 3' untranslated region of the MH class I locus; (b) *Sasa-DAA-3UTR,* a minisatellite marker embedded in the 3' untranslated region of the MH class II alpha locus (Stet et al. 2002; Grimholt *et al.* 2003). In order to distinguish between natural selection and other forces that might impact on genetic variation, we also included eight putatively neutral microsatellite loci in the analysis as controls. Generalised linear models (GLM) were used to test the null hypothesis, i.e. no natural selection, by fitting the observed and expected genotype frequencies to a series of models that included terms representing selection resulting from heterozygote advantage (dominance deviations) or additive allelic effects. The null hypothesis is that under neutrality (i.e. no selection), genotype frequencies in surviving fish would be equal to those expected from parental crosses. The alternative hypotheses would therefore be that either a) frequencies of heterozygotes would be higher than expected in survivors if selection took place as a result of heterozygote advantage, or b) that frequencies of certain alleles (particularly rare ones) would be higher then expected in survivors if negative frequency-dependant selection had taken place. In theory, if a disease mediated selection event had occurred in the river, we would expect no evidence of selection at the eight neutral markers and conversely evidence of selection as a result of either a) or b) at the MH loci.

2. Materials and methods

Hatchery and field methods

The experiment was carried out in a contained section of the Srahrevagh river, located in the Burrishoole catchment in western Ireland (Fig. 1). As it is not acceptable to introduce diseases into pristine environments, we introduced non-native wild fish into the Burrishoole system, which has a history of intensive aquaculture and disease outbreaks including furunculosis (*Aeromonas salmonicida*). We selected wild broodstock from the Owenmore system in Co. Mayo (Fig. 1), approximately 50 km north of the Burrishoole river system, on the basis of *Sasa-DAA-3UTR* and *Sasa-UBA-3UTR* markers, to ensure maximum allelic diversity and heterozygosity. Owenmore fish are not native to the experimental river and thus can be considered naive with respect to many of the pathogens endemic in the Burrishoole system. In addition, there has been no history of aquaculture in the Owenmore system, and thus fish from this system should not have been exposed to any aquaculture-associated diseases.

The Owenmore broodstock were used to produce two groups, with differing allelic composition and expected hatching date, in the Burrishoole hatchery, hereafter called 'Early Owenmore' (eggs and milt stripped from 7 males and 5 females on 18th December 2001) and 'Late Owenmore' (7 males and 4 females on 27th December 2001). A full reciprocal mating system was used, which produced 35 early families and 28 late families, the eggs from which were incubated in the hatchery until eyes were visible ('eyed eggs'). The number of eggs per family ranged from 508 to 1091, with an average of 889. We excluded natural spawners from the experiment river over

the winter of 2001-2002, ensuring that the only salmon hatching in the river in 2002 were from the experiment. This exclusion was facilitated by the presence of a complete upstream and downstream fish trap at the bottom of the Srahrevagh river (Fig. 1). In early February, we counted live eggs, mixed the families and 33,915 Early Owenmore eggs and 22,116 Late Owenmore eggs were planted in the upper reaches of the river in artificial redds (Donaghy & Verspoor 2000) (Fig. 1). Eggs from all families were mixed in one container and divided equally into sixteen egg boxes for placement in the artifical redds, thus ensuring a random distribution of families within the artificial redds. At this eyed egg stage, ten eggs from each family were maintained in the hatchery as controls, to determine hatching success. As the highest level of mortality in natural Atlantic salmon populations occurs in the period following emergence from nests (Einum & Fleming 2000), we quantitatively fished ten sites in the river (Fig. 1) six months after their introduction as eggs (i.e. aged 0+) in order to estimate overall survival and parr distribution over the length of the river. Fish densities were calculated using removal sampling (three passes) (Zippin 1958). We then randomly sampled approximately 1,500 salmon parr from twenty four sections (each approximately 200 metres in length) of the whole river (Fig. 1). Aliquots of tissue from each individual were preserved in 99% ethanol.

Genetic analysis

The immunogenetic loci included in this study were: (a) *Sasa-UBA-3UTR*, a microsatellite marker embedded in the 3' untranslated region of the MH class I locus; (b) *Sasa-DAA-3UTR,* a minisatellite marker embedded in the 3' untranslated region of the MH class II alpha locus (Stet et al. 2002; Grimholt *et al.* 2003). A large number of

population genetics and experimental studies in fish have focussed on the characterization of the MH class II beta locus (Langefors et al. 2001b; Kurtz et al. 2004; Wedekind et al. 2004). The rationale behind this choice is likely the fact that in mammals, class II beta alleles are highly polymorphic (e.g. the HLA-DRB loci with 411 protein alleles), while the associated class II alpha chain is momomorphic (e.g. HLA-DRA with only two protein alleles). However, in salmonids, class II alpha loci are highly polymorphic (Stet *et al.* 2002). Furthermore, class II alpha and class II beta alleles form unique haplotypes (Stet *et al.* 2002): each class II alpha allele is associated with a unique class II beta allele. Therefore, characterization of either the alpha allele or beta allele is sufficient to describe the polymorphism of class II genes. As an initial examination of our results indicated a signature of selection on the *Sasa-DAA-3UTR* minisatellite, we also unambiguously determined the *Sasa-DAA* genotype of all parents and progeny. As the relationship between *Sasa-DAA* alleles or genotypes and *Sasa-DAA-3UTR* markers is not one-to one, in that some of the markers are associated with more than one allele and vice versa, the assignment of *Sasa-DAA* genotype involved the additional typing of an intron length polymorphism in the (linked) MH class II beta (*Sasa-DAB*) locus. *Sasa-DAA/Sasa-DAB* haplotypes have, for the most part, a unique combination of embedded microsatellite markers (H. –J. Megens *et al.*, in prep). For those individuals where *Sasa-DAA* typing using the embedded markers in the *Sasa-DAA* and *Sasa-DAB* genes could not resolve the *Sasa-DAA* allele, DNA sequencing of exon 2 of the *Sasa-DAA* locus was performed according to Consuegra *et al.* (2005). Results from both the MH class II embedded marker *Sasa-DAA-3UTR,* and also the actual MH class II allele *Sasa-DAA* are presented and discussed in the following sections, and are labelled *Sasa-DAA-3UTR* (minisatellite) and *Sasa-DAA* (allele) for clarity.

We typed 836 of the recaptured parr for the *Sasa-UBA-3UTR* dinucleotide microsatellite marker (based on Grimholt *et al.* 2002, but redesigned to obtain much shorter fragments and to avoid null alleles in divergent alleles such as *Sasa-UBA**0901), for the *Sasa-DAA-3UTR* 10 bp minisatellite marker (Grimholt *et al.* 2000; Stet *et al.* 2002), for the *Sasa-DAA* allele and for four control microsatellites (*One*107 (Olsen *et al.* 2000); *Ssa*171, *Ssa*202 (O'Reilly *et al.* 1996); *Ssp*2215 (Paterson *et al.* 2004)) using fluorescently labelled primers. In addition we typed a random subset of 414 fish for an additional four control microsatellites (*Ssa*197 (O'Reilly *et al.* 1996); *Ssa*G7SP (Paterson *et al.* 2004); SSOSL85 (Slettan *et al.* 1995); *Ssa*D144 (King *et al.* 2005)). Samples were split between the two laboratories (Cork and London) and each laboratory typed the full range of loci. A subset of samples was typed by both laboratories to ensure consistency. DNA was extracted using the Wizard SVF Genomic DNA Purification System (Promega) or using a Chelex method (Estoup *et al.* 1996). DNA samples were amplified for all the markers in three multiplex reactions using the Qiagen Multiplex PCR kit in a final volume of 4 µl with 30 cycles of the PCR profile recommended by manufacturers at 58 ºC annealing temperature or in ten independent PCRs (PCR profile consisted of 3min at 95ºC, followed by 30 cycles of 30s at 95ºC, 30s at 56ºC (or 50ºC for MH Class II) and 30s at 72º). Alleles were resolved in a ABI 377 automated sequencer (Applied Biosystems) and allele sizes were evaluated against a TAMRA 350/500 size standards, or on 18 or 25cm 6% polyacrylamide gels using a LiCor 4200 DNA sequencer with allele sizes evaluated against a 50-350bp size standard and a cocktail of common alleles.

Statistical analysis

The statistical analysis was designed to test the null hypothesis that under neutrality (i.e. no selection), genotype frequencies in surviving fish would be equal to those expected from parental crosses. Alternative hypotheses would therefore be that either a) frequencies of heterozygotes would be higher than expected in survivors if selection took place as a result of heterozygote advantage, or b) that frequencies of certain alleles (particularly rare ones) would be higher then expected in survivors if negative frequency-dependant selection had taken place. A generalized linear model (McCullagh & Nelder 1989) was used for the analysis of the data, which was based on the comparison of observed genotype frequencies in fish surviving after 6 months with expected genotype frequencies calculated from parental crosses. This analysis was conducted for each of the eight neutral microsatellite markers, the *Sasa-UBA-3UTR* microsatellite marker, and *Sasa-DAA-3UTR* minisatellite marker, and the *Sasa-DAA* allele. Such models are very similar to standard regression models but are more general in that the response variable can be non-normal (e.g. Poisson or binomially distributed). We assumed that the number of individuals Y_{ij} of each genotype *ij* at a particular locus (the response variable in the model) followed a Poisson distribution, with an expectation of λ_{ij}. Based on the known number of crossings made between parental genotypes, a measure of the expected number of recaptures under neutrality, x_{ij}, was calculated. In the simplest case of neutrality, the observed number of recaptures λ_{ij} should be proportional to expectations based on the crossings made, x_{ij}. Thus, under neutrality we have $\lambda_{ij} = a' x_{ij}$, or, taking logarithmic values,

$$\ln \lambda_{ij} = a + \ln x_{ij}. \qquad (1)$$

This represents our model H00 (see Table 1). Equation (1) is a generalized linear model (McCullagh & Nelder 1989) with a log link-function and a Poisson response variable. This choice of link function ensures that the response variable takes valid values (e.g. only positive values) for all values of the right hand side of equation (1) (the linear predictor of the model). The term $\ln x_{ij,l}$ plays the role of an offset in the model, that is, a covariate for which the regression coefficient is not estimated but instead is known *a priori.*

Different extensions of (1) were considered by including terms representing mechanisms of selection. Firstly, terms representing additive allelic effects s_i and s_j of different alleles *i* and *j* were added to (1) to form model H01. If this model fitted the data well, it would indicate that fish with certain alleles had higher survival than fish with different alleles and that this effect was additive on the log scale. This means that the survival of a particular heterozygote, say *ij*, lies on the arithmetic mean of the survival of the homozygotes *ii* and *jj* on the log scale. Under this model allelic effects on survival are common to both groups of fish (Early Owenmore and Late Owenmore) so that alleles linked with higher survival in the Early Owenmore group are similarly linked to higher survival in Late Owenmore fish. We therefore extended model H01 to include differential survival with additive allelic effects varying between groups *l,* (Early or Late Owenmore) to get model H02:

$$\ln \lambda_{ij,l} = a_l + \ln x_{ij,l} + s_{i,l} + s_{j,l} \ . \qquad (2)$$

The parameters $s_{i,l}$ and $s_{j,l}$ represent the allelic effects of allele *i* and *j* in group *l* respectively, and a_l is an intercept representing average survival in group *l*. In this model, the allelic effects may vary between groups of fish, with, for example, some alleles being linked to higher survival in Early Owenmore fish, but lower survival in Late Owenmore fish.

Secondly, terms representing heterozygote advantage d_h where h=1 for $i \neq j$ (heterozygotes) and h=0 for $i=j$ (homozygotes) were added to the above model alternatives. With the constraint that $d_0 = 0$, the parameter d_1 can thus be interpreted as a common increase (or decrease) in survival of heterozygotes relative to the expectation at the arithmetic mean of the respective homozygotes (the expectation under the model with only additive allelic effects). The advantage of using a common parameter representing heterozygote advantage for all heterozygote types is increased statistical power and a more parsimonious (simpler) model. We also fitted models where all heterozygote advantages d_{ij} were free parameters. Under this model, the survival of at least one heterozygote differs from the expectation under the additive allelic effects model. The different model alternatives that were considered are summarized in Table 1.

The constraint that all allelic effects sum to one was introduced to avoid over-parameterization of the models. The intercept and offset term was included in all models and was fitted using the GLM-function of the software-package R . Models were assessed based on AIC-values (Burnham & Anderson 1998) which were calculated for all model alternatives. Nested model alternatives were also tested against each other using standard tests based on the change in deviance (McCullagh & Nelder 1989). In count data like these, the variance of the response variable is typically larger than expected from the model assumptions, a phenomenon known as over-dispersion (McCullagh & Nelder 1989). To assess this we also computed estimates (McCullagh & Nelder 1989) of the amount of over-dispersion for each selected model, that is, the factor c by which the variance of the response variable exceeds the theoretical variance (the variance is equal to the expectation in case of the

Poisson distribution). Thus, an over-dispersion value close to c=1 indicates that no over-dispersion is present.

The estimates of the allelic effects (parameters s_i and s_j) were used to calculate selection coefficients for each allele. For example, an estimated allelic effect of 0.25 means that an individual carrying one copy of this particular allele on average experiences an increase in survival by a factor of $e^{(0.25)} = 1.28$; that is, a 28% increase in survival or a selection coefficient of 0.28. The survival of individuals carrying two copies of the allele increases by a factor of $e^{(2*0.25)} = 1.64$, i.e. 64% or a selection coefficient of 0.64.

To test for frequency dependent selection we regressed estimated additive allelic effects against allele frequencies, weighing each observation by the precision of the estimate, that is, the inverse of the squared standard error. The non-independence between estimated allelic effects (the response variable in the regression) was ignored, which is likely to inflate the probability of type II errors (false rejection of a true null hypothesis) beyond the nominal 0.05 level.

3. Results

There was minimal hatchery mortality at the egg stage among families (average 1.6% ±3% s.d.), indicating that there was no significant selection in the period up to the eyed-egg stage. In addition, all control eggs retained in the hatchery produced viable fry, indicating a post-hatch success of 100%. From parr density estimates derived from quantitative electrofishing surveys, we estimate that 89% of the progeny introduced into the river died between planting out of eggs and our

sampling, representing a significant potential for selection. Observed genotypes of six of the control microsatellite loci (*One*107, *Ssa*171, *Ssa*197, *Ssp*2215, *Sasa*D144b, SSOSL85) in fish surviving at six months were close to expectations based on neutrality and the genotypes of the parents in the crosses made. Thus, for these loci, the neutral model (H00) was most appropriate (Table 2), as indicated by the low Akaike Information Criterion (AIC) values. For one other control microsatellite locus (*Ssa*G7SP) and the *Sasa-UBA-3UTR* microsatellite marker, the AIC values indicated that the model including common dominance deviations (H10), was a better fit than the neutral model (H00) (Table 2). However, explicit tests of H00 vs. H10 were not significant in both cases (p=0.12 and p=0.09 for *Sasa-UBA-3UTR* and *Ssa*G7SP loci respectively), even without correcting for multiple comparisons, making it unlikely that selection had acted at these loci. For *Ssa*202, the H01 model had a lower AIC value than the neutral model (H00), and the difference was significant (H00 vs H01, D=22.4, df=10, p=0.013). This indicates that there was selection at this locus. The occurrence of alleles of all loci in eggs (predicted from the genotypes of parents) and in fish surviving at 6 months can be found in the supplementary material (Table S1).

Analysis of the *Sasa-DAA-3UTR* marker and *Sasa-DAA* allele data showed that observed genotypes in fish surviving at 6 months deviated significantly from genotypes that would be expected from the parental cross (Fig. 1), indicating that selection had occurred at these loci. The occurrence of *Sasa-DAA* alleles in eggs and fish surviving at 6 months are given in Table 3. The AIC values for both the *Sasa-DAA-3UTR* marker and *Sasa-DAA* allele indicate that model H01 was the best fit for the data (Table 2) which indicates that the mechanism of selection was an additive allelic effect common to both groups of fish (Early and Late Owenmore). Analysis of the deviance of the various models indicated that the difference in the allelic effects

between groups was only marginally statistically significant (H01 vs. H02, D=17.05, df=9, p=0.048) and that dominance deviations were only significantly different from zero when main additive allelic effects were not present (H00 vs. H20, *D*=324, df=661, $p<0.00001$), and were not statistically significant when additive allelic effects were included (H01 vs. H21, *D*=53, df=52, *p*=0.44). An extension of the selected model, which included only a common dominance deviation, was also not significant (H01 vs. H11, *D*=0.09, df=1, *p*=0.76). Estimates of the allelic effects under the selected model (H01) are given in Table 4. Selection coefficients (calculated from the allelic point estimates) ranged from –0.96 to 0.96 (Fig. 2), indicating average survival could decrease by up to 96% or increase by up to 96%, depending on the *Sasa-DAA* allele that a fish was carrying. This value was calculated for heterozygote fish, and the selection coefficient would be significantly higher for homozygote fish.

There was no significant relationship between overall allelic effects and frequencies for the *Sasa-DAA* allele (t=0.59, df=11, *p*=0.57).

4. Discussion and conclusions

In our experiment on the relative fitness of genotypes during early juvenile life in Atlantic salmon, the data for both the marker and allele *Sasa-DAA* (MH class II) loci deviated significantly from neutrality, while the data for seven out of eight control microsatellites and the *Sasa-UBA-3UTR* (Class I) locus did not. The *Ssa202* microsatellite showed a signal of selection, and in retrospect, may not have been a good choice as a putative neutral marker as this marker has been recently shown to be sex-linked (Woram et al. 2003; Gilbey et al. 2004). As natural selection resulting from disease should only be detectable at immunogenetic loci, such as MHC, while other

forces such as genetic drift, migration and mutation should be detectable at both control and immunogenetic loci (Garrigan & Hedrick 2003), we conclude that the cause of the deviation of the *Sasa-DAA* loci from neutrality was related to selection on the MH class II as a result of an immune response, rather than any demographic event. Our results also show that additive allelic effects were more important than heterozygote advantage (dominance deviation) at this locus in determining juvenile survival. This supports the suggestion of Grimholt *et al.* (2003) that homozygosity may be an advantage for MH class II in laboratory based disease challenge experiments, and also concurs with several studies of MHC selection in natural vertebrate populations, which found evidence for balancing selection through frequency dependence rather than heterozygote advantage (Paterson et al. 1998; Meyer-Lucht & Sommer 2005; Schad et al. 2005).

Additive allelic effects are consistent with the hypotheses that frequency-dependent selection or fluctuating selection pressures from pathogens maintain MHC polymorphism more so than heterozygote advantage. Although we found alleles that were either positively or negatively associated with survival, our results do not permit us to conclusively state whether the mechanism involved is frequency-dependent selection as there was no significant relationship between overall allelic effects and frequencies. This is contrary to the results for other free-ranging vertebrates such as Soay Sheep (Paterson *et al.* 1998) and Gray mouse lemurs (Schad *et al.* 2005), which found that common alleles were associated with reduced survival. In light of these results, it would seem that Hedrick's model (Hedrick 2002), which suggests that temporal variability in pathogen resistance (fluctuating selection pressure) can maintain MHC polymorphism in the absence of frequency dependent selection, may be applicable to wild salmon populations.

While MH class I and class II loci were included in this experiment, only the data from MH class II showed evidence of selection. This result is not unexpected for a number of reasons. MHC class I gene products are specifically involved in the presentation of virus-derived peptides leading to the activation of cytotoxic T cells that eliminate virus-infected cells. In contrast, MHC class II gene products have a broader spectrum of action as they present pathogen-derived peptides to T helper cells, leading to the activation of phagocytic cells and the production of antibodies, immunological traits that are involved in the elimination of parasites and bacteria and neutralization of viruses (Janeway *et al.* 2005). The relative importance of class II traits over class I traits is underpinned by the difference of persistence over evolutionary time-scales of allelic lineages of class I and class II genes in salmonids (Shum *et al.* 2001) and is also evidenced by observations on the distribution of class I and class II alleles among sympatric species of the Lake Tana large African barb species flock (Kruiswijk *et al.* 2005). In the latter, it was observed that sympatric species of the large African barbs share class I alleles, but the class II alleles are completely partitioned among the different sympatric barb species. Significantly, results from populations of Atlantic salmon in the Baltic sea indicated that a MH class II alpha locus departed significantly from neutrality, while a MH class I locus did not, suggesting that the MH class II locus was under balancing selection (Vasemägi *et al.* 2005). Finally, it should also be noted that the data on the MHC class I locus was actually on variation at the *Sasa-UBA-3UTR* microsatellite marker embedded in the 3' untranslated region of the gene. Although the relationship between allele size at *Sasa-UBA-3UTR* and sequence variation at the MHC class I locus was consistent in a Norwegian farmed population (Grimholt et al. 2002), it is possible that this relationship is not as consistent in the natural population studied here as a result of

recombination and/or mutation. Using variation at *Sasa-UBA-3UTR* may therefore have reduced our power to detect selective effects on the MHC class I locus.

There was a very small, but statistically significant, amount of over-dispersion in the data, typically around c=1.2 (Table 2), suggesting that some explanatory variable influencing survival was not accounted for by the chosen models. One possibility is a hitchhiking effect, where selection on the *Sasa-DAA* locus may lead to apparent selection on other linked and unlinked loci, unless there is perfect linkage disequilibrium (Crow & Kimura 1970), something that is unlikely in practice when crossings are made between finite numbers of parents. A family effect, that is, differing fitness of individual alleles in the different genetic backgrounds inherited from each parent, may be another possibility. In designing our experiment we endeavoured to maximise the number of available alleles as there was no *a priori* knowledge of what alleles might be important to the survival of fish in the wild, even though we were cognisant that a large number of parents may compromise the statistical sensitivity of the model to examine family effects (sire effect, dam effect and sire x dam interaction) in addition to allelic effects (due to overparameterisation). This proved to be the case in our analysis, and we were unable to successfully model family effects with the data available. However, the small amount of over-dispersion in the study suggests that such possible family or hitchhiking effects were only of minor importance. Consequently, it appears reasonable to ignore dependencies between genotype counts from the same families, which should also be small. A future experiment, either typing significantly greater numbers of survivors than were analysed here or using a reduced number of parents would allow an analysis of the interaction between parental and allelic effects.

Given the history of furunculosis, a disease caused by the bacterium *Aeromonas salmonicida,* in the Burrishoole system, which Owenmore fish would not have been previously exposed too, we would have expected to see some correlation between the *DAA* alleles identified by Grimholt *et al.* (2003) as being resistance or susceptible to furunculosis and the results from our experiment. This proved not to be the case however, as two of the alleles (*Sasa-DAA*0501* and *Sasa-DAA*0101*) which were found to be associated with susceptibility to furunculosis in the lab, or at least a variant of that disease, had higher relative fitness in our fish. This suggests that our experimental population either did not experience a furunculosis epidemic, or if they did, it was a different strain to that used in the laboratory experiment. Alternatively, this result may indicate that, while laboratory studies can highlight which MH alleles can confer resistance or susceptibility to a particular pathogen in a small experimental population (Langefors et al. 2001a; Lohm et al. 2002; Grimholt et al. 2003), it may be that the same allele cannot be considered advantageous or disadvantageous in a wild population in a natural environment with a wide range of pathogens varying over space and time.

As MHC class II molecules have a broad spectrum of activity, we have no indication of what the pathogen or pathogens might have been in this experiment. We assume that the Burrishoole river system has a unique community of naturally occurring endemic pathogens, which is likely to be different from the pathogen community in the Owenmore River. The MH class II allele composition of the Owenmore fish is a product of the host-pathogen relationship in the native river, evolving over a period of time i.e. local adaptation. In transferring the Owenmore fish to a new environment in the Burrishoole system, we potentially exposed the fish to new selective pressures to which they were not adapted. This experimental design is

one way of exposing animals to novel disease challenges. While the most obvious way of doing this would be to directly introduce specific pathogens into the wild, and monitor the response in the recipient population, our experimental design, transferring the animal to the pathogen, has an obvious advantage from an ethical point of view, but lacks specificity in determining the selective agent.

Given the potential for variability in pathogens and pathogenicity over time and space in the wild, it would seem that natural or hatchery populations have the best chance of dealing with episodic and variable disease challenges if MHC genetic variation is preserved both among and within populations. This is particularly relevant to wild Atlantic salmon as they are increasingly at risk of losing genetic variation either directly through introgression with cultured fish (McGinnity *et al.* 2003), or indirectly as a result of disease transmission from aquaculture (Håstein & Lindstad 1991; Johnsen & Jensen 1994; McVicar 1997). While it might be argued that the introduction of novel genes and parasites from aquaculture stocks is exactly the sort of raw material that is needed to maintain and increase genetic diversity, we would argue that such introgressions have the potential to bring in a range of pathogens, but not a lot of new alleles, particularly in the case of MH class II. This conclusion is supported by genetic typing work on Norwegian farm fish (Stet et al. 2002; Consuegra et al. 2005), which failed to find MH alleles in farm fish that were not already present in their wild counterparts, and in addition found a smaller number of alleles in the farm populations. With respect to hatchery populations, there is currently considerable interest within large commercial breeding programs in identifying specific genes associated with disease resistance. However, as populations that have been cultivated in hatcheries often show loss of genetic variability due to the effects of inbreeding (Norris *et al.* 1999), our results suggest that substantial benefits in

disease resistance could be conferred by simply reducing the effects of inbreeding and maintaining allelic variation at MH loci.

Our conclusions are based on the early life stages of Atlantic salmon. However, it is worth noting that this species undergoes a number of other major mortality events, both prior to smolting, and in the sea. Further work is required to quantify the impact of MH genotype on survival of salmon over their entire life history and thus its contribution to overall Darwinian fitness.

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References

Bernatchez, L. & Landry, C. 2002 MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? *Journal of Evolutionary Biology* **16**, 363-377.

Burnham, K. P. & Anderson, D. R. 1998 *Model Selection and Multimodal Inference. A Practical Information-Theoretic Approach*. New York: Springer.

Consuegra, S., Megens, H., Leon, K., Stet, R. J. M. & Jordan, W. C. 2005 Patterns of variability at the major histocompatibility class II alpha locus in Atlantic salmon contrast with those at the class I locus. *Immunogenetics* **57**, 16-24.

Donaghy, M. J. & Verspoor, E. 2000 A new design of instream incubator for planting out and monitoring Atlantic salmon eggs. *North American Journal of Fisheries Management* **20**, 521-527.

Einum, S. & Fleming, I. A. 2000 Selection against late emergence and small offspring in Atlantic Salmon (*Salmo salar*). *Evolution* **54**, 628-639.

Estoup, A., Largiader, C. R., Perrot, E. & Chourrout, D. 1996 Rapid one-tube extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* **5**, 295-298.

Garrigan, D. & Hedrick, P. W. 2003 Perspective: Detecting adaptive molecular polymorphism: lessons from the MHC. *Evolution* **57**, 1707-1722.

Gilbey, J., Verspoor, E., McLay, A. & Houlihan, D. 2004 A microsatellite linkage map for Atlantic salmon (*Salmo salar*). *Animal Genetics* **35**, 98-105.

Grimholt, U., Drabløs, F., Jørgensen, S. M., Høyheim, B. & Stet, R. J. M. 2002 The major histocompatability class I locus in Atlantic salmon (*Salmo salar* L.): polymorphism, linkage analysis and protein modelling. *Immunogenetics* **54**, 570-581.

Grimholt, U., Getahun, A., Hermsen, T. & Stet, R. J. M. 2000 The major histocompatibility class II alpha chain in salmonid fishes. *Developmental and Comparative Immunology* **24**, 751-763.

Grimholt, U., Larsen, S., Nordmo, R., Midtlyng, P., Kjoeglum, S., Storset, A., Saebø, S. & Stet, R. J. M. 2003 MHC polymorphism and disease resistance in

Atlantic salmon (*Salmo salar*); facing pathogens with single expressed major histocompatibility class I and class II loci. *Immunogenetics* **55**, 210-219.

Håstein, T. & Lindstad, T. 1991 Diseases in wild and cultured salmon: possible interactions. *Aquaculture* **98**, 277-288.

Hedrick, P. W. 2002 Pathogen resistance and genetic variation at MHC loci. *Evolution* **56**, 1902-1908.

Hedrick, P. W., Lee, R. N. & Garrigan, D. 2002 Major histocompatibility complex variation in red wolves: evidence for common ancestry with coyotes and balancing selection. *Molecular Ecology* **11**, 1905-1913.

Hedrick, P. W., Parker, K. M., Miller, E. L. & Miller, P. S. 1999 Major histocompatibility complex variation in the endangered Przewalski's horse. *Genetics* **152**, 1701-1710.

Hughes, A. L. & Nei, M. 1989 Nucleotide substitution at major histocompatability complex class II loci: Evidence for overdominant selection. *Proceedings of the National Academy of Science of the United States of America* **86**, 958-962.

Hughes, A. L., Yeager, M. & Carrington, M. 1996 Peptide binding function and the paradox of HLA disease associations. *Immunology and Cell Biology* **74**, 444-448.

Janeway, C. A., Travers, P., Walport, M. & Shlomchik, M. 2005 *Immunobiology: The immune system in health and disease*. New York: Garland Publishing.

Johnsen, B. O. & Jensen, A. J. 1994 The spread of furunculosis in salmonids in Norwegian rivers. *Journal of Fish Biology* **45**, 47-55.

King, T. L., Eackles, S. & Letcher, B. H. 2005 Microsatellite DNA markers for the study of Atlantic salmon (*Salmo salar*) kinship, population structure, and mixed-fishery analyses. *Molecular Ecology Notes* **5**, 130.

Klein, J. 1986 *The Natural History of the Major Histocompatibility Complex*. New York: Wiley & Sons.

Kruiswijk, C. P., Hermsen, T., van Heerwaarden, J., Dixon, B., Savelkoul, H. F. & Stet, R. J. M. 2005 Major histocompatibility genes in the Lake Tana African large barb species flock: evidence for complete partitioning of class II B, but not class I, genes among different species. *Immunogenetics* **56**, 894-908.

Kurtz, J., Kalbe, M., Aeschlimann, P. B., Häberli, M. A., Wegner, K. M., Reusch, T. B. H. & Milinski, M. 2004 Major histocompatibility complex diversity influences parasite resistance and annate immunity in sticklebacks. *Proceedings of the Royal Society of London, B.* **271**, 197-204.

Langefors, Å., Lohm, J., Grahn, M., Andersen, Ø. & von Schantz, T. 2001a Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. *Proceeding of the Royal Society of London Series B Biological Sciences* **268**, 479-485.

Langefors, Å., Lohm, J. & von Schantz, T. 2001b Allelic polymorphism in MHC class II B in four populations of Atlantic salmon (*Salmo salar*). *Immunogenetics* **53**, 329-336.

Lohm, J., Grahn, M., Langefors, Å., Andersen, Ø., Storset, A. & von Schantz, T. 2002 Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection. *Proceeding of the Royal Society of London Series B Biological Sciences* **269**, 2029-2033.

Marsh, S. G. E., Parham, P. & Barber, L. D. 2000 *The HLA factsbook*. San Diego: Academic Press.

McClelland, E. E., Adler, F. R., Granger, D. L. & Potts, W. K. 2004 Major histocompatibility complex controls the trajectory but not host-specific

adaptation during virulence evolution of the pathogenic fungus *Cryptococcus neoformans*. *Proceedings of the Royal Society of London, B.* **271**, 1557-1564.

McClelland, E. E., Granger, D. L. & Potts, W. K. 2003 Major histocompatibility complex-dependent susceptibility to *Cryptococcus neoformans* in mice. *Infection and Immunity* **71**, 4815-4817.

McCullagh, P. & Nelder, J. A. 1989 *Generalized Linear Models*. London: Chapman & Hall.

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Ò Maoileidigh, N., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J. & Cross, T. 2003 Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 2443-2450.

McVicar, A. H. 1997 Disease and parasite implications of the coexistence of wild and cultured Atlantic salmon populations. *ICES Journal of Marine Science* **54**, 1093-1103.

Meyer-Lucht, Y. & Sommer, S. 2005 MHC diversity and the association to nematode parasitism in the yellow necked mouse (*Apodemus flavicollis*). *Molecular Ecology* **14**, 2233-2243.

Naylor, R., Hindar, K., Fleming, I. A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D. & Mangel, M. 2005 Fugitive Salmon: assessing the risks of escaped fish from net-pen aquaculture. *Bioscience* **55**, 427-437.

Norris, A. T., Bradley, D. G. & Cunningham, E. P. 1999 Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* **180**, 247-264.

Olsen, J. B., Kretschmer, E. J., Wilson, S. L. & Seeb, J. E. 2000 Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. *Molecular Ecology* **9**, 2185-2187.

O'Reilly, P. T., Hamilton, L. C., McConnell, S. K. & Wright, J. M. 1996 Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2292-2298.

Paterson, S., Piertney, S. B., Knox, D., Gilbey, J. & Verspoor, E. 2004 Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (Salmo salar L.) microsatellites. *Molecular Ecology Notes* **4**, 160-162.

Paterson, S., Wilson, K. & Pemberton, J. M. 1998 Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (Ovis aries L.). *Proceedings of the National Academy of Science of the United States of America* **95**, 3714-3719.

R Development Core Team. 2004 *R: A Language and Environment for Statistical Computing*. Vienna.

Roberge, C., Einum, S., Guderley, H. & Bernatchez, L. 2006 Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. *Molecular Ecology* **15**, 9-20.

Schad, J., Ganzhorn, J. U. & Sommer, S. 2005 MHC constitution and parasite burden in the Malagasy mouse lemur, *Microcebus murinus*. *Evolution* **59**, 439-450.

Shum, B. P., Guethlein, L., Flodin, L. R., Adkison, M. A., Hedrick, R. P., Nehring, R. B., Stet, R. J. M., Secombes, C. & Parham, P. 2001 Modes of Salmonid MHC Class I and II evolution differ from the primate paradigm. *Journal of Immunology* **166**, 3297-3308.

Slettan, A., Olsaker, I. & Lie, Ø. 1995 Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics* **26**.

Sommer, S. 2005 The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology* **2**, 16-34.

Stet, R. J. M., de Vries, B., Mudde, K., Hermsen, T., van Heerwaarden, J., Shum, B. P. & Grimholt, U. 2002 Unique haployptes of co-segregating major histocompatibility class II A and class II B alleles in Atlantic salmon (*Salmo salar*) give rise to diverse class II genotypes. *Immunogenetics* **54**, 320-331.

Vasemägi, A., Gross, R., Paaver, T., Koljonen, M.-L., Säisä, M. & Nilsson, J. 2005 Analysis of gene associated tandem repeat markers in Atlantic salmon (*Salmo salar* L.) populations: implications for restoration and conservation in the Baltic Sea. *Conservation Genetics* **6**, 385-397.

Wedekind, C., Walker, M., Portmann, J., Cenni, B., Muller, R. & Binz, T. 2004 MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *Journal of Evolutionary Biology* **17**, 11-18.

Woram, R. A., Gharbi, K., Sakamoto, T., Hoyheim, B., Holm, L.-E., Naish, K., McGowan, C., Ferguson, M. M., Phillips, R. B., Stein, J., Guyomard, R., Cairney, M., Taggart, J. B., Powell, R., Davidson, W. & Danzmann, R. G. 2003 Comparative Genome Analysis of the Primary Sex-Determining Locus in Salmonid Fishes. *Genome Res.* **13**, 272-280.

Zippin, C. 1958 The removal method of population estimation. *Journal of Wildlife Management* **22**, 82-90.

Figure 1. Locations of the Burrishoole and Owenmore catchments in Ireland (top left panel), the experiment river in the Burrishoole catchment (bottom left panel) and quantitative electrofishing sites, electrofishing sections for sample collection and artifical redds in the experiment river (right panel).

Figure 2. *Sasa-DAA* (MH Class II) allele frequencies in Atlantic salmon eggs (grey bars) and 0+ parr surviving after 6 months in a natural river (white bars) for two groups of fish – Early Owenmore, (top) and Late Owenmore (bottom). Egg allele frequencies were calculated from parental crosses.

Figure 3. Selection coefficients for *Sasa-DAA* (MH Class II) alleles in Atlantic Salmon surviving after 6 months in a natural river. The selection coefficient for a heterozygote = $e^{(\text{allelic point estimate})} - 1$. Allelic point estimates were calculated by fitting observed and expected genotype frequencies to model H01 (see Table 1 for details).

Short title: Natural selection on MH in wild salmon

Table 1. Summary of alternative selection models fitted using GLM to observed and expected genotype frequencies in Atlantic salmon surviving in the wild in a section of the Burrishoole river system six months after introduction. A full explanation of the models used is described in the *Statistical analysis* section of Materials and methods.

	neutrality	$+ d_{\mathrm{H}}$ (common dominance deviation)	$+ d_{ij}$ (separate dominance deviations)
neutrality	H00	H10	H20
$+ s_i + s_j$ (additive allelic effects)	H01	H11	H21
$+ s_{i,l} + s_{j,l}$ (different allelic effects between groups)	H02	H12	H22

Table 2. AIC-values and estimates of *c* (over-dispersion) of 9 model alternatives (see Table 1 for more detail) for each locus typed in Atlantic salmon surviving in the wild in a section of the Burrishoole river system six months after introduction. The lowest AIC (Akaike Information Criterion) value (in bold) are conisdered to be the best fit for that locus.

locus	n	H00	H10	H20	H01	H11	H21	H02	H12	H22	*c*
*Ssa*197	414	**785.1**	786.2	845.1	791.2	792.9	852.0	796.3	798.1	857.5	1.25
*Ssa*D144b	414	**737.6**	739.2	814.2	738.7	740.7	819.5	756.5	758.5	835.0	1.13
*Ssa*G7SP	414	791.3	**790.5**	815.6	792.9	793.3	816.5	800.5	801.0	822.4	1.11
SSOSL85	414	**735.1**	736.7	785.4	743.6	745.5	794.1	754.1	756.1	805.7	1.07
*Ssa*202	836	805.5	807.4	854.5	**803.1**	804.9	862.7	811.5	813.4	870.4	1.07
*Ssp*2215	836	**853.0**	854.7	903.3	857.5	859.5	906.8	866.4	868.4	914.9	1.13
*One*107	836	**966.0**	967.9	1091.3	979.4	981.4	1093.4	988.0	989.9	1100.9	1.10
*Ssa*171	836	**907.8**	909.5	942.5	913.2	915.2	946.0	923.0	925.0	955.4	1.19
Sasa-UBA-3UTR	836	962.1	**961.7**	1029.5	964.1	964.5	1027.3	970.3	970.2	1025.2	2.06
Sasa-DAA-3UTR	836	1005.7	1006.9	968.8	**935.8**	936.6	960.9	936.7	936.6	958.3	1.92
Sasa-DAA allele	836	1067.2	1069.2	980.1	**922.9**	924.8	974.0	923.9	925.4	979.1	1.07

Table 3. Number of occurrences of *Sasa-DAA* (MH class II) alleles in eggs (expected allele frequency calculated from parental genotypes) and parr surviving in river after 6 months (observed allele frequency) in two experimental groups of Atlantic salmon. Total number of eggs introduced into the river was 56031, and the number of surviving parr typed was 836 (for this locus, n=797 fish or 1594 alleles) as 39 fish had ambiguous MHC II alleles).

Sasa-DAA allele	No. of occurrences in eggs	Frequency (expected)	No. of occurrences in parr	Frequency (observed)
*DAA*0101*	8293	0.07	119	0.07
*DAA*0201*	10937	0.10	184	0.12
*DAA*0301*	3997	0.04	71	0.04
*DAA*0302*	11406	0.10	245	0.15
*DAA*0303*	2050	0.02	1	0.00
*DAA*0304*	7884	0.07	55	0.03
*DAA*0401*	9672	0.09	125	0.08
*DAA*0501*	4024	0.04	64	0.04
*DAA*0601*	27621	0.25	360	0.23
*DAA*0602*	3488	0.03	57	0.04
*DAA*0901*	5549.5	0.05	115	0.07
*DAA*1001*	6168	0.06	55	0.03
*DAA*1202*	10972.5	0.10	143	0.09

Table 4. Allelic point estimates (±standard errors) and selection coefficients for each allele at the *Sasa-DAA* locus calculated using Model H01. An estimated allelic point effect of, for example, 0.25 means that an individual carrying one copy of this particular allele on average experiences an increase in survival by a factor of $e^{(0.25)} = 1.28$; that is, a 28% increase in survival or a selection coefficient of 0.28.

Sasa-DAA allele	allelic point estimate	standard error	selection coefficient
*DAA*0101*	0.31	0.12	0.36
*DAA*0201*	0.46	0.11	0.59
*DAA*0301*	0.53	0.14	0.70
*DAA*0302*	0.67	0.10	0.96
*DAA*0303*	-3.20	0.93	-0.96
*DAA*0304*	-0.41	0.15	-0.33
*DAA*0401*	0.11	0.12	0.12
*DAA*0501*	0.42	0.15	0.52
*DAA*0601*	0.17	0.10	0.19
*DAA*0602*	0.35	0.15	0.41
*DAA*0901*	0.61	0.13	0.84
*DAA*1001*	-0.22	0.02	-0.20
*DAA*1202*	0.20	0.13	0.23

Figure 1.

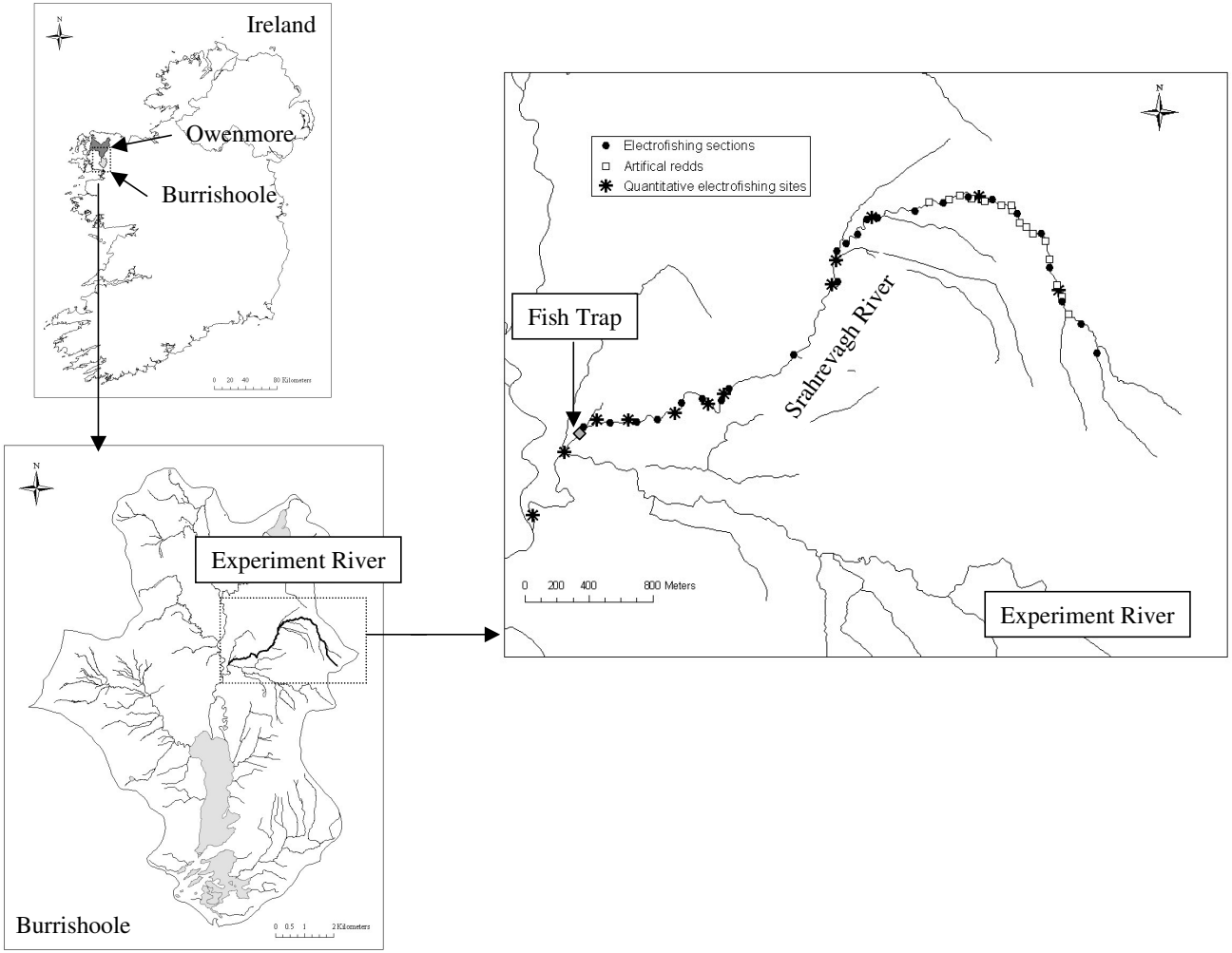
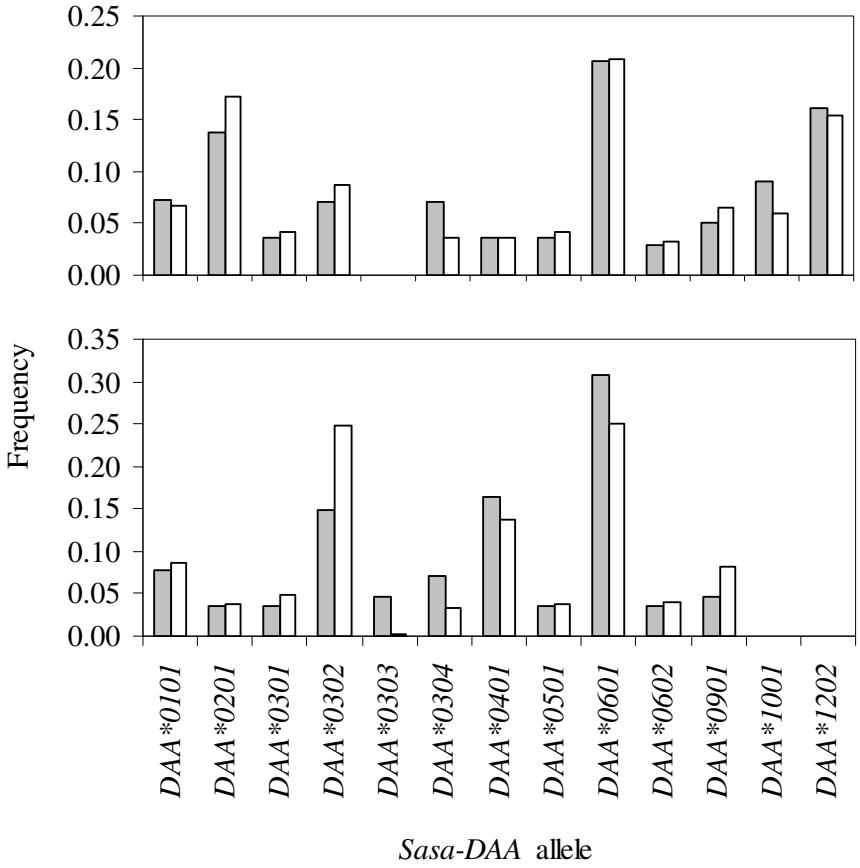

Ireland
Owenmore
Burrishoole
Experiment River
Burrishoole
Electrofishing sections
Artifical redds
Quantitative electrofishing sites
Fish Trap
Srahrevagh River
0 200 400 800 Meters
Experiment River

Figure 2.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Frequency
DAA*0101
DAA*0201
DAA*0301
DAA*0302
DAA*0303
DAA*0304
DAA*0401
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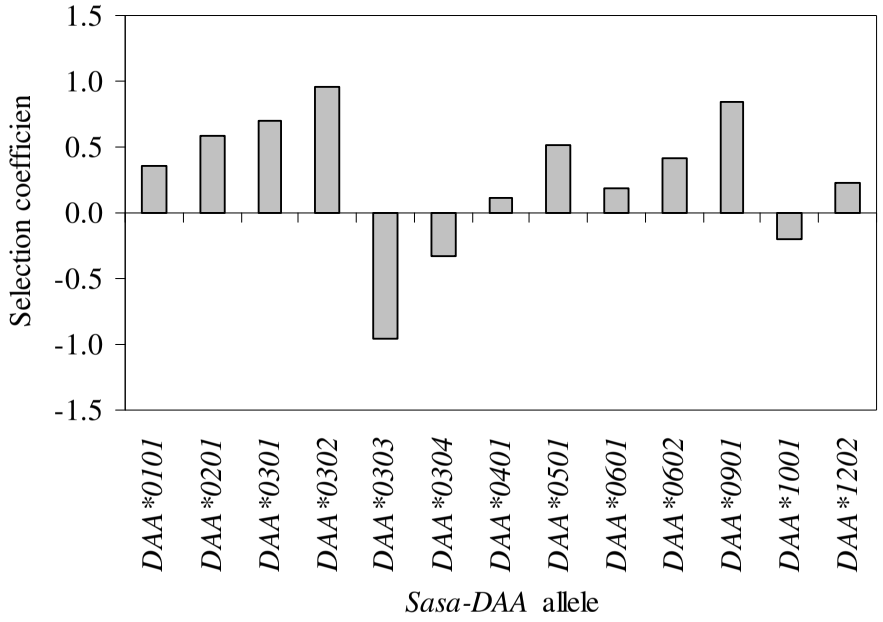
DAA*0901
DAA*1001
DAA*1202
Sasa-DAA allele

Figure 3.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Selection coefficien
DAA*0101
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DAA*0602
DAA*0901
DAA*1001
DAA*1202
Sasa-DAA allele